

FILE 'MEDLINE' ENTERED AT 14:29:14 ON 19 AUG 2002

FILE 'AGRICOLA' ENTERED AT 14:29:14 ON 19 AUG 2002

FILE 'CAPLUS' ENTERED AT 14:29:14 ON 19 AUG 2002
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FILE 'WPIDS' ENTERED AT 14:29:14 ON 19 AUG 2002
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=> s (urokinase or upa!) and (sp (w) sepharose) and benzamidine
L1 2 (UROKINASE OR UPA!) AND (SP (W) SEPHAROSE) AND BENZAMIDINE

=> d 1- ibib abs
YOU HAVE REQUESTED DATA FROM 2 ANSWERS - CONTINUE? Y/(N):y

L1 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:344480 CAPLUS
DOCUMENT NUMBER: 133:131555
TITLE: Construction and characterization of two mutants of
pro-urokinase (Ala175.fwdarw.Ser,
Tyr18.fwdarw.His and Ala175.fwdarw.Ser, Tyr187.fwdarw.
His)
AUTHOR(S): Shi, Wei; Zhu, Hui; Xue, Yu-ming; Tang, Tang; Ma,
Zhong
CORPORATE SOURCE: Department of Biochemistry, National Key Laboratory of
Pharmaceutical Biotechnology, Nanjing University,
Nanjing, 210093, Peop. Rep. China
SOURCE: Nanjing Daxue Xuebao, Ziran Kexue (2000), 36(2),
208-212
CODEN: NCHPAZ; ISSN: 0469-5097
PUBLISHER: Nanjing Daxue
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB Human single chain urokinase-type plasminogen activator (scu-PA, also named pro-UK) is an important thrombolytic agent in therapy of thrombosis. The activation of plasminogen on the surface of fibrin induced by pro-UK, is much specific and effective, resulting very small tendency of bleeding from system lytic state. Therefore, great importance has been attached to scu-PA in clinics. Though pro-UK has some selectivity for fibrin, its higher selectivity for fibrin in human bodies was counteracted by its higher intrinsic activity when it was used in large doses. The risk of bleeding still remains. It was discovered that the stretch of 297.apprx.313 amino acids in scu-PA formed a loop. Lys300, the only pos. charge amino acid in the loop might interact with Asp355 which was sited near the active center by charge attraction. Thus Ser365 was pulled, Ser365 Asp225, His204 might form the active center on the 3-D structure resulted in the high intrinsic activity of pro-UK. Besides, according to the data of crystal structure of chymotrypsin, Asp194, His40and Ser32 formed a zymogen triad, which keeps the inactive conformation of chymotrypsin. As a member of the zymogens of serine protease family, pro-UK lacks the zymogen triad, His40 and Ser32 were replaced resp. by Tyr187 and Ala175. This was also the structure basis of pro-UK's high enzymic activity. To reduce the intrinsic activity of pro-UK, 2 mutant genes of pro-urokinase, muk1 (Ala175.fwdarw.Ser, Tyr187.fwdarw.His, Lys300.fwdarw.His) and muk2 (Ala175.fwdarw.Ser, Tyr187.fwdarw.His) were constructed by site directed mutagenesis, and were expressed in E. Coli BL21. The expressed inclusion body was treated by denaturation and renaturation, and purified by SP-sepharose ion-exchange chromatog. and Benzamidine Sepharose affinity adsorption. Using the synthetic substrate S2444, the intrinsic activity and the enzymic activity of two-chain form of muk1 and muk2 were measured. The intrinsic activities of muk1 and muk2 were 8-fold and 2.5-fold lower than that of pro-urokinase, resp. The enzymic activity of two-chain muk1 was 1.5-fold higher than that of urokinase and the activity of muk2 was the same as wild type urokinase. The mechanism and the structure basis of a much higher intrinsic catalytic activity than other zymogens of the serine protease family was discussed.

L1 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2000:141655 CAPLUS

DOCUMENT NUMBER: 133:39761

TITLE: Construction and characterization of a mutant of single-chain urokinase-type plasminogen activator (Ser175-His187-mscu-PA)

AUTHOR(S): Xue, Yu-Ming; Zhu, Hui; Shi, Wei; Liu, Wei; Liu, Jian-Ning; Ma, Zhong

CORPORATE SOURCE: Department of Biochemistry, National Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing, 210093, Peop. Rep. China

SOURCE: Shengwu Huaxue Yu Shengwu Wuli Xuebao (2000), 32(1), 26-30

CODEN: SHWPAU; ISSN: 0582-9879

PUBLISHER: Shanghai Kexue Jishu Chubanshe

DOCUMENT TYPE: Journal

LANGUAGE: Chinese

AB Single-chain urokinase-type plasminogen activator (scu-PA) is the precursor of double-chain urokinase (tcu-PA), which has a much higher intrinsic catalytic activity than other zymogens of the serine protease family. To restore the "zymogen triad" of Asp-His-Ser in the serine protease family, the mutant gene of scu-PA (mscu-PA, Ala175 .fwdarw. Ser175, Tyr187 .fwdarw. His187) was constructed by the method of oligonucleotide-directed, site-specific mutagenesis in order to reduce its intrinsic catalytic activity. mscu-PA was expressed in E. coli BL21. After denaturation and renaturation in vitro, the mscu-PA was purified to homogeneity by SP-Sepharose ion-exchange chromatog., Sephacryl S-200 chromatog. and Benzamidine-Sepharose affinity adsorption, and the mutant mscu-PA had the same activity to plasmin as scu-PA. The catalytic efficiency (measured by kcat/Km) of the mutant to synthetic substrate S2444 was 2.5-fold lower than that of scu-PA, and the activity against Glu-plasminogen was also reduced. After activation by plasmin, mscu-PA and tcu-PA had similar catalytic efficiency against S2444 and Glu-plasminogen. The intrinsic catalytic activity of mscu-PA may be reduced after restoring the "zymogen triad".

PCT/US02/08853 SEARCH RESULTS/HISTORY

(FILE 'HOME' ENTERED AT 15:21:57 ON 19 AUG 2002)

FILE 'MEDLINE, AGRICOLA, CAPLUS, BIOSIS, EMBASE, WPIDS' ENTERED AT
15:22:01 ON 19 AUG 2002

L1 2 S (UROKINASE OR UPA!) AND (SP (W) SEPHAROSE)

FILE 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,
BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT,
CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DGENE, DRUGB,
DRUGLAUNCH, DRUGMONOG2, DRUGNL, DRUGU, DRUGUPDATES, .' ENTERED AT
15:23:12 ON 19 AUG 2002

INDEX 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,
BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA,
CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB,
DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 15:23:27 ON
19 AUG 2002

SEA (UROKINASE OR UPA!) AND (SP (W) SEPHAROSE)

2 FILE CAPLUS
1 FILE SCISEARCH
2 FILE TOXCENTER
27 FILE USPATFULL

L2 QUE (UROKINASE OR UPA!) AND (SP (W) SEPHAROSE)

FILE 'USPATFULL' ENTERED AT 15:24:20 ON 19 AUG 2002

L3 0 S (UROKINASE OR UPA!) (A) (SP (W) SEPHAROSE)

L4 27 S (UROKINASE OR UPA!) AND (SP (W) SEPHAROSE)

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